tightly correlated with emission of 22 kHz calls (Figure 1D). This is expected as call emission is mechanistically linked with breathing [5,7]. Accordingly, rats maintained long uninterrupted exhalations to accommodate continuous emission of prolonged 22 kHz calls (Figure 1A bottom; Figure S2A). Thus, the observed changes in sniff-cycle duration following face-to-face interactions were mirroring the increases in 22 kHz calls (Figure 1E). Sniff duration also tracked the time course of 22 kHz emission following face-to-face interactions, with both measures peaking 1 second following interactions and returning to baseline after 5 seconds (Figure S2B).

Overall, sniff cycles with 22 kHz calls were approximately five times longer than non-22 kHz sniffs during social interactions (Figure 1F). This clear separation by duration makes it possible to identify the emission of 22 kHz calls based on sniff cycle duration alone. To determine whether 22 kHz calls were likely present in the data reported in Wesson [1], we directly measured the duration of sniffs from the four example panels illustrating decreases in sniff rate following face-to-face interactions. As expected, sniffs reported for dominant rats were short and thus incompatible with 22 kHz call production (Figure S2C). For submissive rats, however, we estimate that 41% of the reported sniffs had 22 kHz calls based on their long duration (Figure 1G; and see Supplemental Experimental Procedures). It is therefore likely that the large reductions in sniff rate following face-to-face interactions in Wesson [1] were due to disruption of sniffing behavior by 22 kHz vocal production.

What aspects of rat behavior communicate dominance during social interactions? Wesson [1] showed that sniffing patterns correlate with submissive status during face-to-face encounters. 22 kHz calls are known to signal dominance over long timescales. As expected, submissive rats could be reliably identified by their general emission of 22 kHz calls. Our finding that these calls are rapidly triggered after face-to-face encounters further implicates this signal in communication at faster timescales. During ultrasound emission, rats make prolonged exhalations against a constricted larynx [7], reflected in our intranasal recordings as periods of flat signal. Mechanistically, sniffing and vocal output carry redundant information. Rats are known to perceive and react to the loud (60–80 dB) 22 kHz alarm calls [8], making these salient signals unlikely to be ignored by conspecifics. Even so, sniffing and vocalization are only part of a larger ensemble of correlated behaviors comprising social interactions, including also whisking and postural changes [9, 10]. Disentangling the effects of each individual behavior would require targeted manipulations. Together with Wesson [1] and Wolfe *et al.* [10], our studies demonstrate that face-to-face encounters are relevant discrete events in the establishment of social dominance.

Supplemental Information

Supplemental Information includes experimental procedures and two figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.10.007.

Acknowledgments

Funding was from the Leon Levy Foundation. We thank Pawel Wojcik for help with sniffing sensor design and Aniruddha Das and Dmitry Rinberg for helpful comments.

References

1. Wesson, D. W. (2013). Sniffing behavior communicates social hierarchy. Curr. Biol. *23*, 575–580.
2. Sales, G. D. (1972). Ultrasound and aggressive behaviour in rats and other small mammals. Anim. Behav. *20*, 88–100.
3. Thomas, D.A., Takahashi, L.K., and Barfield, R.J. (1983). Analysis of ultrasonic vocalizations emitted by residents during aggressive encounters among rats (Rattus norvegicus). J. Comp. Psychol. *97*, 207–212.
4. Anderson, JW. (1954). The production of ultrasonic sounds by laboratory rats and other mammals. Science *119*, 808–809.
5. Roberts, L.H. (1972). Correlation of respiration and ultrasound production in rodents and bats. J. Zool. *168*, 439–449.
6. Lore, R., Flannelly, K., and Farina, P. (1976). Ultrasounds produced by rats accompany decreases in intraspecific fighting. Aggress. Behav. *2*, 175–181.
7. Riede, T. (2011). Subglottal pressure, tracheal airflow and intrinsic laryngeal muscle activity during rat ultrasound vocalization. J. Neurophysiol. *106*, 2580–2592.
8. Brudzynski, S.M. (2001). Pharmacological and behavioral characteristics of 22 kHz alarm calls in rats. Neurosci. Biobehav. Rev. *25*, 611–617.
9. Barnett, S.A. (1975). The Rat: A Study in Behavior (Chicago: University of Chicago Press).
10. Wolfe, J., Mende, C., and Brecht, M. (2011). Social facial touch in rats. Behav. Neurosci. *125*, 900–910.

Shelby White and Leon Levy Center for Brain, Mind and Behavior, The Rockefeller University, New York, NY 10065, USA.
*E-mail: ysirotin@rockefeller.edu, dlaplagne@rockefeller.edu (equal contributors)

Response to Assini *et al.*

Daniel W. Wesson

Animals have evolved behavioral and neural specializations which are optimal for survival within their respective physical environments. I recently proposed [1] that socially interacting rats may monitor each other's sniffing through perception of subtle auditory and/or tactile cues given off by the act of sniffing and further hypothesized the "likely possibility that reciprocal sniffing behavior coincides with other established forms of communication (ultrasonic vocalizations, odor emission) that together allow optimal intraspecific communication". Assini *et al.* [2] provide a timely test of this hypothesis, and in doing so, show that reciprocal sniffing behavior [1] and ultrasonic vocalizations coincide in their display by submissive rats. Here I attempt to frame these two supportive studies together with the goal of a greater understanding of animal social cognition and communication.

Building upon work by pioneers in the rodent behavior community (too many to cite, but see [3]), recent studies have provided detailed insights into the complex social displays of sensorimotor behaviors among rats [1,2,4]. In my recent study [1], I directly recorded the sniffing behaviors of rats during social interactions. I found that rats display reciprocal exchanges of face-to-face sniffing which are dependent upon the social status of the rat, independent of odor sampling, and impact future displays of agonistic behavior [1]. During face-to-face encounters, dominant rats increased their sniffing frequency and concomitantly, subordinate rats decreased theirs. Failure of the subordinate rats to decrease their sniffing frequency was correlated with a shortened latency for aggressive behaviors by dominant rats.

On the basis of this finding, I proposed that rats may monitor each other's sniffing through perception of subtle auditory and or tactile cues given off by the act of sniffing [1]. I also hypothesized the "likely possibility that reciprocal sniffing

behavior coincides with other established forms of communication (ultrasonic vocalizations, odor emission) that together allow optimal intraspecific communication". This view also received support from Bennett Galef [5] in the same issue of *Current Biology*. Assini *et al.* [2] sought to test this hypothesis and showed that reciprocal sniffing and ultrasonic vocalizations coincide in their display by submissive rats, and that, in some (but not all) cases, ultrasonic vocalizations may impact the display of sniffing.

Assini *et al.* [2] have replicated my finding of reciprocal sniffing behavior [1] and extended this by demonstrating that 22 kHz ultrasonic vocalizations, well-known to be displayed in a variety of contexts [6], coincide with the display of reciprocal respiratory behavior. I have no substantial disagreements with their results, or their conclusions. I would, however, like to address a few assertions which the authors make which I believe are essential for our understanding of social communication in rats, but also in other animals.

Rodents, including rats, are known to display a wide variety of behaviors for both intra- and inter-specific communication [3]. Ultrasonic vocalizations are just one among many possible communication signals discovered to date that are displayed by rats. Assini *et al.* [2] have suggested that 22 kHz ultrasonic vocalizations are an important communication tool because they are 'salient'. The authors imply that this 'salient' signal may dominate over other proposed communication-related signals used by rats during proximal interaction (for example, sniffing [1] and whisking [4]). Yet we should all ask ourselves, salient to whom? The human experimenter thinking that they too would respond more rapidly to a loud, discrete stimulus? Or to the rat who is already face-to-face, only a few millimeters apart, and need not make nor hear a loud stimulus to orchestrate its next behavioral response? The use of the word 'salient' by Assini and colleagues places unwarranted importance upon a signal (more precisely a behavior). While humans, like many primates, may rely strongly upon auditory cues [7], this is not the case among rodents, which in fact have greater reliance upon other modalities (such as olfaction) [3].

The evidence by Assini *et al.* [2] that ultrasonic vocalizations and respiration are linked in rats does not prove that ultrasonic vocalizations are more important than other possible cues during social interactions. Cues emitted by the act of proximal sniffing (whether mechanosensory or auditory) may still be important in navigating social encounters. Both sniffing and ultrasonic vocalizations may, however, be of subsequent importance to yet another signal. Perhaps evidence for this resides in the fact that Assini *et al.* [2] found that not all displays of reciprocal sniffing coincided with ultrasonic vocalization emission by subordinate rats — reflecting that in some cases subordinate rats negotiate approach by dominant rats in manners independent of ultrasonic vocalization emission (perhaps through sniffing [1] or whisking [4]). Supporting this, original work on ultrasonic vocalizations emission in a similar paradigm reported that subordinate rats generally do not emit ultrasonic vocalizations [8].

Both my previous study [1] and that of Assini *et al.* [2] place an index of intent upon the behavioral displays of animals which was not quantified. In other words, both studies claim an animal is displaying a behavior without knowing whether that is the sole, necessary and sufficient, mediator of the display. This is notably a difficult task to accomplish in the context of rodent social behaviors, since any experimental manipulation may directly impact the display or utility of other behaviors. In my study [1], I proposed that subordinate rats may reduce their respiratory frequency during face-to-face social interactions to deescalate a potentially conflicting encounter by a dominant rat. While it is clear that this respiratory behavior may serve as a quantitative index of conflict avoidance, the observation of reciprocal sniffing and my finding that it correlated with the latency to the next agonistic behavior by dominant animals, however, did not prove this was the *motivation* of this behavior. Indeed, numerous behavioral displays have been interpreted as driven by communicative purposes, but in fact serve and are perhaps motivated by physiological needs (for example, [9]).

Nagel [10] argued that it is nearly impossible for us humans to truly understand what it is like to be another animal due at least in part to our inability to understand the 'subjective character' of experience. In that same logic, it is hard for us as humans to consider how rats, *en-soi*, communicate and utilize sensory information during their social encounters to navigate and survive these critical situations. Relevant to the discussed studies, communicative mechanisms for social appeasement and conflict avoidance in rats are not well understood and future research, with careful attention to assigning intent, will be needed to clarify how environment and possibly previous social experience shape behavioral displays of communication signals among rats.

Acknowledgments

I would like to thank the four anonymous reviewers of this manuscript for thoughtful correspondences which greatly improved the substance of this piece. This work was supported by NSF grant IOS-1121471.

References

1. Wesson, D.W. (2013). Sniffing behavior communicates social hierarchy. Curr. Biol. *23*, 575–580.
2. Assini, R., Sirotin, Y., and Laplagne, D. (2013). Vocalizations disrupt sniffing following social interactions. Curr. Biol. *23*, R996–R997.
3. Wolff, J.O., and Sherman, P.W. eds. (2007). Rodent Societies: An Ecological & Evolutionary Perspective (Chicago: University of Chicago Press).
4. Wolfe, J., Mende, C., and Brecht, M. (2011). Social facial touch in rats. Behav. Neurosci. *125*, 900–910.
5. Galef, B.G. (2013). Animal communication: sniffing is about more than just smell. Curr. Biol. *23*, R272–R273.
6. Portfors, C.V. (2007). Types and functions of ultrasonic vocalizations in laboratory rats and mice. J. Am. Ass. Lab. Anim. Sci. *46*, 28–34.
7. Ghazanfar, A.A. ed. (2003). Primate Audition: Ethology and Neurobiology (Boca Raton, FL: CRC Press).
8. Thomas, D.A., Takahashi, L.K., and Barfield, R.J. (1983). Analysis of ultrasonic vocalizations emitted by intruders during aggressive encounters among rats (Rattus norvegicus). J. Comp. Psychol. *97*, 201–206.
9. Blumberg, M.S., and Alberts, J.R. (1990). Ultrasonic vocalizations by rat pups in the cold: an acoustic by-product of laryngeal braking? Behav. Neurosci. *104*, 808–817.
10. Nagel, T. (1974). What is it like to be a bat? Philos. Rev. *83*, 435–450.

Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106, USA.
E-mail: dww53@case.edu